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# Growth and Care Instructions of a New Model Species—the Lycophyte *Selaginella apoda*

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ABSTRACT.—*Selaginella apoda* has many advantages as a model species, including a short life cycle. In order to work effectively with a lycophyte model species, it is important to be able to grow the gametophytes and sporophytes quickly. In the case of *S. apoda*, the gametophytes grow quickly and have high fertilization success in warm nutrient enriched water. Sporophytes at the three root stage can be easily transplanted to soil. They grow well in moist containers at a wide range of temperatures. Treatment with 0.15% Previcur®N prevents most fungal contaminations.

KEY WORDS.—model organism, lycophytes, lycopods, ferns, cultivation

*Selaginella apoda* (L.) Spring has many advantages as a model species, including an 85 day life cycle from spore to spore (Schulz *et al.*, 2010). The length of the life cycle is similar to other model species, such as *Ceratopteris thalictroides* (L.) Brongn., with a 3.5 month life cycle (Klekowski, 1970). In order to work effectively with a model species, it is important to be able to grow it quickly. In addition, a high frequency of fertilization is desirable.

Many different methods of growing ferns have been published (e.g., Lovis, 1968; Hickok and Warne, 2004), but only a small amount is known about growing *Selaginella*. Methods for growing gametophytes and sporophytes of *Selaginella* have been described, but the conditions and treatments vary among species. Bierhorst (1964) collected megaspores from the soil around *Selaginella* sporophytes and placed them on wet filter paper, where the spore wall opened. For the microgametophytes, he sowed whole microsporangia on filter paper. Bold (1967) sowed spores on plaster of Paris blocks half immersed in distilled water or an inorganic medium. If the plaster blocks remained sufficiently moist, fertilization would occur. Overall, little is known about growing *S. apoda*, therefore, we have undertaken this investigation to present an instruction on how to grow gametophytes and sporophytes of *S. apoda*.

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## Materials and Methods

The specimens investigated here were collected from a naturally occurring population on the grounds of the New York Botanical Garden (population voucher: *Little & Moran 935*, NY). We grew specimens in the Nolen Greenhouse (New York, USA), in the greenhouse at the Botanical Garden of the Ruhr-University Bochum (Germany), and in laboratory conditions both in New York (USA) and Bochum (Germany). We grew *Selaginella apoda* six times through its entire life cycle. Spores were soaked in autoclaved water for 4 minutes, surface sterilized for 4 minutes, and then rinsed with autoclaved water for 4 minutes. For surface sterilization, the fungicides Benomyl (1–20 mg/L), sodium hypochlorite (0.1–0.875%), Previcur®N (propamocarb–hydrochlorite) (0.15%), and ethanol (70%) were tested. In addition, the antibiotic streptomycin (5–60 mg/L) was tested. Soil, sandy soil, peat soil, autoclaved water, autoclaved water with nutrients (C-fern nutrients) and C-fern agar (Carolina) were each used as media. The composition of these nutrient mixtures was the same as used by Klekowski (1969).

## Results and Discussion

There are, theoretically of course, many possible protocols to grow *S. apoda* under different conditions. The method presented here is just one possibility, but it is very fast and successful. Masses of megaspores and microspores can be collected from pots of *S. apoda* (sporophytes with cones) by placing the pots on their sides on top of a smooth collecting surface (we used a sheet protector; Avery Denniso, Brea, CA; Fig. 1A). The greatest number and largest cones appeared in late summer and autumn (in New York). The cones produced in spring and early summer are fewer in number and much shorter. Presumably, cone and, therefore, spore production are influenced by day length and light intensity, but we have not investigated these parameters independently. In order to harvest a greater volume of spores, let the plants dry for one day during the collection, being careful not to dry the plants so much that they die. The collected spores can be stored in dry vials at room temperature; viability is maintained for at least two years. Growth can be accelerated by soaking the spores in water (or nutrient water) for 15 minutes before sowing.

Spore surface sterilization was necessary to grow sterile gametophyte cultures on used agar (Fig. 1 B, C). Biological contamination rarely occurred when soil or autoclaved water with or without nutrients was used as a medium; however, surface sterilization was necessary when using C-fern agar as a substrate. It is important to soak the spores with autoclaved water and to rinse the spores with autoclaved water after the treatment. Even a low concentration of 0.1% sodium hypochlorite, as routinely used for *Ceratopteris* spores (Hickok and Warne, 2004), killed the *S. apoda* megaspores. Sodium hypochlorite prevented only a small amount of fungal contamination. Both low concentrations of Benomyl and 70% ethanol also killed megaspores and prevented only some of the fungal contamination. In contrast, 0.15%

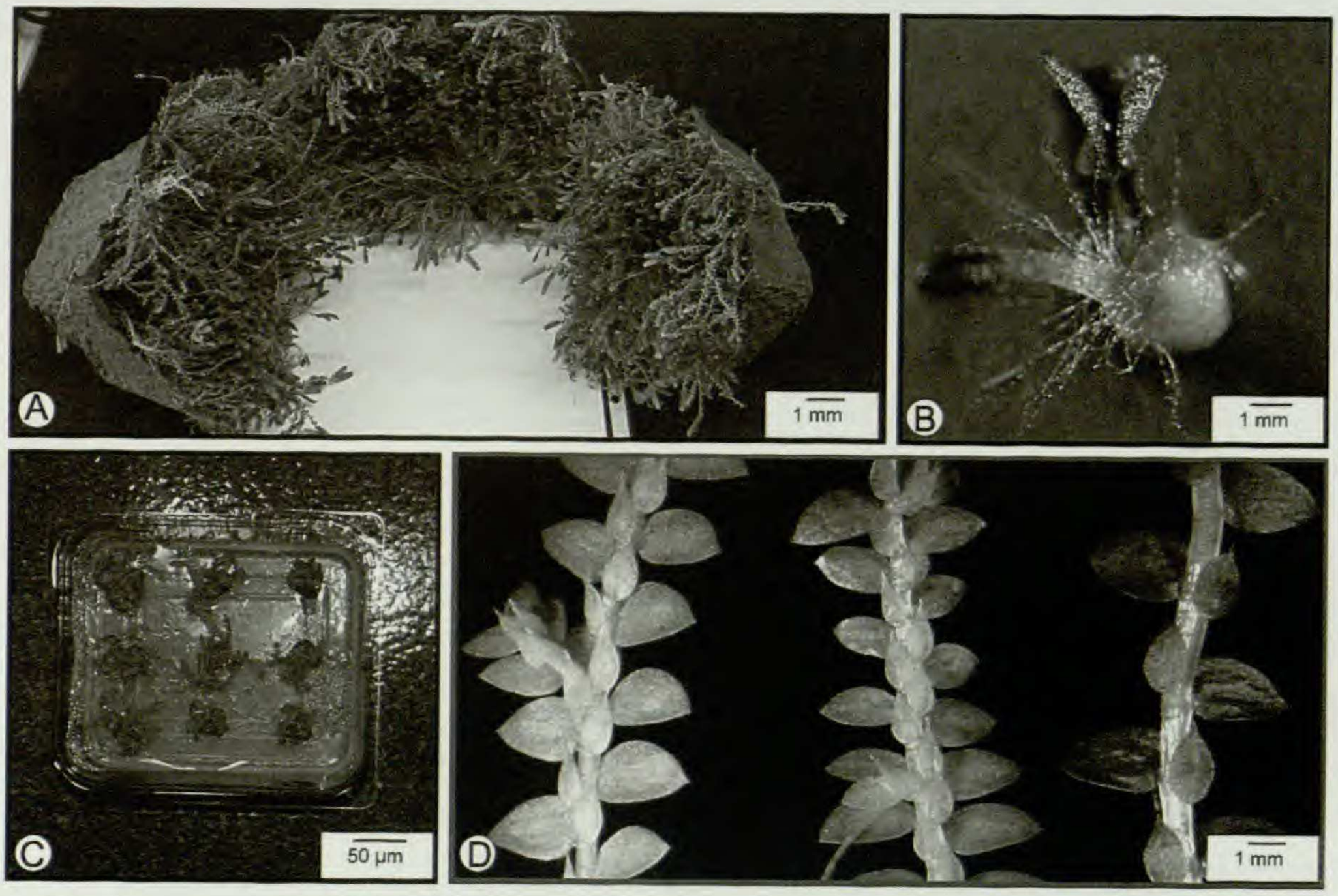


FIG. 1. *Selaginella apoda*. A: the pots with sporophytes arranged laterally on a smooth surface to collect mega- and microspores. B: young sporophyte growing on agar with Previcur®N. C: growth of sporophytes in a culture box. D: left: sporophytes growing in a bright place; the leaves are almost white; middle: sporophytes growing in a darker place than the sporophyte left but in a lighter place than the sporophyte right, the leaves are light green; right: sporophytes growing in a half shady place, the leaves are green.

Previcur®N prevented most fungal contaminations without killing the megaspores. Rarely, bacterial contamination occurred on C-fern agar, but 15 mg/L streptomycin (4 min) prevented such contamination. In order to prevent contamination with algae, it was sufficient to remove megaspores with algae (easily observed as green dots) from the culture.

The fertilization success varied with substrate. We observed the highest fertilization success (about 95%) in autoclaved water with nutrients. In autoclaved water and on C-fern agar (60–80%) fertilization was less frequent. On soil, the fertilization success was moderate (about 60%: Table 1).

An important factor for gametophyte development is temperature (Webster, 1967). The optimum temperature for gametophytic development is about 30°C. Similar growth and development of gametophytes can be obtained at 22–30°C. The higher the temperature within this range, the faster the gametophytes develop. Temperatures lower than 22°C will alter or inhibit the developmental timing. Light is not necessary for the development of the gametophytes. Gametophytes develop successfully under a 14h light/10h dark cycle as well as without any light (24h dark cycle) and in/on different kinds of media/ substrates.

TABLE 1. Number of developing sporophytes on different substrate/medium after different surface sterilization.

| Substrate/ medium | Surface sterilization | Development | Fungal contamination | Bacterial contamination | Fertilization success |
|---|---|---|---|---|---|
| soil | none | ++ | − | − | + |
| water | none | ++ | − | − | ++ |
| water with nutrients | none | +++ | + | + | +++ |
| water with nutrients | 0.15% Previcur®N | +++ | − | − | +++ |
| C–fern agar | none | ++ | +++ | +++ | + |
| C–fern agar | 0.1% sodium hypochlorite | − | ++ | ++ | − |
| C–fern agar | 70% ethanol | − | + | + | − |
| C–fern agar | 5 mg/L Benomyl | − | + | + | − |
| C–fern agar | 0.15% Previcur®N | ++ | − | (+) | +(+) |
| C–fern agar | 0.15% Previcur®N, 15 mg/L streptomycin | ++ | − | − | +(+) |

+ = 1–20%, ++ = 21–60%, +++ = 60–100%, (+) = 1–5%, +(+) = 10–40%

Webster (1979) described *S. apoda* growing in finely sifted sandy loam. Bruchmann (1912) indicated that peat is not suitable for the growth of *Selaginella* gametophytes as it has an inhibiting effect on *S. denticulata* (L.) Spring. However, we were able to grow *S. apoda* on peat soil (e.g., on peat pellets), on mineral agar (e.g., C-fern agar), in mineral medium (e.g., C-fern medium) or in tap water. For experimental purposes, a single megaspore and a single microspore develop very well in a 200 µL tube with 20 µL of water. It is important that the gametophytes are always covered with a film of warm water in order to prevent retardation of development and for fertilization success (the spermatozoids must be able to swim to the egg cells). If young sporophytes are grown in deep water, they usually produce elongated hypocotyls.

The optimal stage to transplant young sporophytes grown on agar (Fig. 1C) or in medium to soil is when the first three roots and several leaves are evident. The mineral nutrient medium used for gametophytes is not optimum for sporophytic growth, so transfer to a soil medium is preferable. Soil should be kept moist to wet at all times. Peat soil or sandy peat works well for *S. apoda*. The plants require warm temperatures (15–28°C) and high humidity (70–100%) to grow quickly.

The sporophytes grew very well at temperatures between 15°C and 28°C. The sporophytes grew faster at higher temperatures and produced more roots and rhizophores. Room temperature works fine for sporophytes. *Selaginella apoda* naturally lives in partially shaded habitats without direct sun. The more light the plants receive, the more the leaves turn yellow/white (i.e., are photobleached: Fig. 1D), and the faster the cones develop. With too much light, the plants will die. The less light the plants receive, the greener and smaller the leaves become. The optimal habitat is shady. Sporophytes grow fast in peat soil, and a bit slower on agar or in (nutrient) water ($n > 100$). If the

plants grow in soil, it is useful to use clay pots and keep the soil wet. The sporophytes need high humidity, otherwise the leaves dry out and the plant will die. The plants can be grown easily in a covered terrarium. The fungicide 0.15% Previcur®N (propamocarb–hydrochlorite) worked well against fungal contamination; it also works well with other *Selaginella* species. High concentrations of other fungicides can damage the plants.

In summer or under artificial light, when plants are growing very fast, fertilizer may be used. A low concentration of fertilizer is required for some *Selaginella* species. We achieved good growth and cone production with Compo® Orchid fertilizer (3–4–5–0.9 N–P–K–micronutrience) [Comp GmbH & Co. KG, Münster]. The recommended concentration should be applied once a month.

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